Tritium in the environment and its impact assessment against the existing radiation protection framework revisited.

N. Jain[1] and A. L. Bhatia[2]
[1]Department of Zoology, Government College, Chimanpura (Jaipur) Rajasthan, India
[2]Department of Zoology, University of Rajasthan, Jaipur, India.

Abstract. With the development of nuclear power programme, an additional consideration outside the existing radiation protection framework appeared: the need to assess not only the level of exposure, but also to take into consideration the accidents which could release large inventories of radioactivity. With the expansion of nuclear commitments, the inventories of Tritium are bound to increase. The possible use of tritium as the fuel for fusion reactors in the near future may result in an additional source of tritium. Swiss albino mice of 1, 2, 3, 4 & 6 weeks of age were injected with tritiated water (HTO) at the dose 111 kBq/gram body weight and the animals from each age group autopsied on 1, 7 and 30 days post- injection. The mouse show radiovulnerability with a capability to repair and recover from the rendered damage during the first half (1 week to 3 week) of postnatal development, whereas during the second half (4 week to 6 week of age) a tendency towards radioresistance is achieved The presentation is an attempt to revisit the behaviour of tritium with its possible implications on the environment in accordance with the presently projected radiation protection framework.

1. INTRODUCTION

Nuclear power programmes have no doubt been affected by the Three Miles Island, the, Chernobyl and most recently by the Fukushima Daiichi disasters. After the March 11, 2011 earthquake, at one instance, the Fukushima plant was releasing 10,000 terabecquerels of Iodine- 131 for several hours. Level 7 accidents are defined as releasing tens of thousands of terabecquerels. International Nuclear and Radiological Event Scale. (INES) created in 1989 by the International Atomic Energy Agency (IAEA) and the Nuclear Energy Agency of the Organization for Economic Co-operation and Development, ranges from 1 (anomaly) to 7 (major accident). The scale is intended to make the public aware about the seriousness of a nuclear event, an accident classified as having a major radioactive release with widespread impact on the environment and public health. In spite of all this, some countries will continue with their well established programmes, while others are expected to suffer a delay in their plants for nuclear power development. It is gratifying to note that the nuclear safety technology itself envisages warding off the potential danger from concentrated radiotoxins in such large quantities. Emission of tritium from the nuclear fuel cycle will increasingly become the dominant source of this nuclide and can become more important than the residue from weapons testing.

Emissions from operating light water reactors are, and will continue to be, insignificant as compared to the releases from proposed fuel reprocessing.

In recent years heated controversy has developed concerning the health and environmental impact of nuclear reactors producing tritium and other radioactive by products which may in the final analysis end up as world-wide contaminants. Tritium is the heaviest and the only radioactive isotope of hydrogen. It decays to the stable isotope $_3He$ by emitting low energy electrons (β-rays): $_3H^1$-$_3He^2$. The short range and low energy of β-rays give rise to almost complete absorption of the radiation energy within the biological matter. Consequently, the effects of $_3H$ are localized and the external radiation hazards from $_3H$ are considered to be negligible. Tritium is widely distributed throughout the mans environment because of its ubiquitous form as tritiated water and its persistence in the environment [1]. Its production by natural processes was discovered by Libby [2]. It is produced naturally in the upper atmosphere by the interaction of Cosmic rays with Nitrogen and Hydrogen. The tritons in the upper atmosphere are oxidised to tritiated water (HTO) and mix with the hydrosphere generally through the movement of air masses and precipitation. Terrestrially, tritium may be formed by the action of Lithium on neutrons.
Incorporation of tritium from tritiated water in mammalian brain has been studied by several investigators, but its radiobiological effects have been ignored. Brain is heavily exposed due to more specific activity of organic tritium and its slow turnover. In the development of mouse cerebellum, neurogenesis and migration of different cell types occurs during first few weeks after birth. Proliferative and premigrative cells are highly radiosensitive during this phase of rapid development [3-4]. Certain radiobiological effects of tritiated water on mouse brain and radiopathological changes in the developing mouse cerebellum have been reported recently [5-7]. Present investigation is an attempt to access the impact of tritium on biological systems together with an analysis on its behaviour in the environment.

1.1 Materials and methods

In order to evaluate the age related cerebellar vulnerability due to acute HTO exposure during postnatal development, mice belonging to different age groups viz: 1, 2, 3, 4, 5 and 6 weeks were injected with HTO at the dose 111kBq/gm body weight. The animals from each group were autopsied on 1, 7 and 30 days *post- injection.* The animals were sacrificed by cervical dislocation and the brains were removed, weighed and fixed in Bouin’s fixative. Cerebellum from each of the fixed brains was removed by cutting the cerebellar peduncles and then processed for making Paraffin blocks. Sections of cerebellar tissue 5µm thick were passed through a graded series of alcohol and stained in Eosin and Haematoxylin for histological examinations. Qualitative studies in the treated lots were compared with the control groups.

*1.1.**1 Dosimetry**:* When the dose give has been single, the Initial Dose Rate (IDR) is calculated. The IDR is defined as the rate at which the energy is imparted to unit mass of

tissue and in the present investigation it has been calculated to be 0.0092Gy/day when the animals were injected with 111kBq/gm body weight HTO.

2. RESULTS

Swiss albino mice of 1, 2, 3 4, 5 and 6 weeks of age were injected with tritiated water (HTO) at the dose 111 kBq (3.0 µCi)/gram body weight and the animals from each age group autopsied on 1, 7 and 30 days post- injection and hence, qualitatively and quantitatively studied for cerebellar vulnerability (Figs. 1-7) The cerebellum consists of three cytoarchitectural layers: 1. Molecular layer 2. Granular layer and 3. The Purkinje cell layer. Of the three cerebellar layers, Purkinje cell layer was most radiosensitive which suffered the most at all intervals when treated

with 111kBq dose of HTO. Radioresponding behaviour of Purkinje cells of 1 and 2 week old age groups of mouse cerebellum reflects a maximum reduction in their number on 7th day post-injection followed by a recovery and reparability phase on 30th day post-exposure.

Qualitative studies reveal that the cerebellum suffers from more radiopathological lesions till 3 weeks of postnatal development, 7 days post HTO-exposure in form of reduced number of Purkinje cells, vacuolation in the molecular layer, enhanced pyknosis and necrosis in the granular and molecular layer cells and displacement of Purkinje cells into the molecular leading into the formation of empty basquets which on 30th day p. i. repairs and recovers. With the advent of age, many DARK TYPE PURKINJE CELLS containing more LIPOFUSCIN (the aging pigment) and accounting for an accelerated aging make their appearance together with the LIGHTER ONES post- irradiation.

3. DISCUSSION

There has been a considerable apprehension that tritium constitutes a unique hazard because a small amount of tritium introduced as tritiated water finds its way through metabolic pathways into the newly synthesised DNA [1]. The transfer of tritium of organic compounds following intake of tritiated water occurs largely by exchange of tritium from body water with the labile hydrogen of organic fractions. About 30% of the total body hydrogen is in tissue solids. In animals continuously exposed to environmental tritium, tritium will equilibrate with the exchangeable hydrogen throughout the body. With prolonged intake of tritiated water, the specific activity of tissue bound hydrogen has risen to as high as 35% of that of the body water. On the contrary, a single tritiated water exposure leads to an initial fixation in tissue solids of about 1-2% of the amount given. In rats and mice exposed to tritiated water for extended periods, the highest relative concentrations were in the brain lipids [8]; followed by skin and muscle [9].

In a general sense, hydrogen bounded to oxygen, nitrogen, sulphur or phosphorous will readily exchange with tritium in water, whereas, hydrogen bounded to carbon is usually not exchangeable except during enzyme mediated reactions. It has been observed with pulsed exposures that the brain of the mouse is somewhat slower to accept free water

tritium into the organic fraction than are other tissues, except for the body lipids and lungs, but that the half-life within those tissues is markedly longer than the average for the entire mouse [10]. This supports the general conclusion that slow uptake is logically associated with long retention. Due to retention of HTO after prolonged exposure, brain seems to be one of the critical organs.

An approximate assessment of risk of tritium oxide ingested during pregnancy by mammals has been demonstrated where brain has been found to be most affected due to more specific activity of organic tritium and its slow turnover [11]. Neuroblasts and stem cells present in mouse brain foetuses are highly radiosensitive and are killed due to exposure. Cell death induction by low HTO doses in the developing cerebellar cortex reported in the present study confirmed earlier findings of Cerda [12]. Apart from it, cell necrosis and pycnosis have also been observed in the present study. Such radiation effects could arise from the death at mitosis of glia, neuronal precursors, both, or by killing of post- mitotic but immature neurons. These could also stem from an intrusion on migration through an alteration of cell surface phenomenon or through death of glia cells that guide the migratory neurons. Cellular events in developing rat cerebellum exposed to X- rays on day 17^{th} of gestation have been studied earlier [13]. Similar results have been observed in the present study. Recent reports on the effects of low doses of HTO on the developing mouse cerebellum by Jain and Bhatia [14-15] support the hypothesis that even low doses of HTO impart a significant deleterious effect on the cerebellum of Swiss albino mouse as has been seen in the present investigation.

4. CONCLUSION

Tritiated water (HTO) exposure appears to impart significant effects on the cerebellum of Swiss albino mice during its various developmental stages. In cerebellum, where the cell renewal system is lacking, major cytoarchitectural changes occur mainly during the first three weeks after birth. This accounts for its high radiovulnerability *vis-à-vis* a capability to repair and recover from the rendered damage during the first half (1 week to 3 week) of postnatal development, whereas during the second half (4 week to 6 week of age) a tendency towards radioresistance is achieved. As the age advances and the animal approaches maturity, relatively lesser post- irradiation impairments become evident. With the advent of age after HTO-exposure many dark type Purkinje cells make their appearance along with the light type. The dark type Purkinje cells biochemically evaluated have been shown to possess more lipofuscin than the lighter ones, specifically only at higher doses. Radiotoxic effects of low doses of tritium on cerebellum are not consistent and apparently quite higher with those expected from an equivalent absorbed dose from external X- irradiation. Therefore, in comparison to its currently projected level of unity, the possibility of a higher RBE of tritium from HTO with regard to brain and especially cerebellum can not be safely ruled out. When it comes to nuclear safety

technology in general, according to NCRP, doses from tritium and nuclear plant effluents are negligible contribution to the background radiation to which people are generally exposed and they account for less than 0.1% of the total background dose. However, recent nuclear disaster at Fukushima, Japan poses a serious concern. According to a current report from Japan's Nuclear and Industrial Safety Agency (NISA), the amount of radioactive material being released at Fukushima reached to a level less than 1 terabecquerel. The environmental group announced that it found radiation levels equivalent to an annual exposure of 5 millisieverts. Due to such a release, there is every possibility of acute and delayed health effects over a wide area, possibly involving many countries together with long-term environmental consequences. The U.N. too had estimated a long-term death toll due to such an exposure. Though, raising Fukushima's level to 7 may not trigger any immediate worsening of nuclear set up over the globe, however, it is sure to add to the publics' growing concern on nuclear safety technology and management over what could happen at dozens of other such nuclear reactors spread across the seismic zones. Hence, the debate is again open to form a consensus on the issue: Whether radiation is still a safe, clean and most potent tool for energy production!!!!

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Fig.1

Fig.2

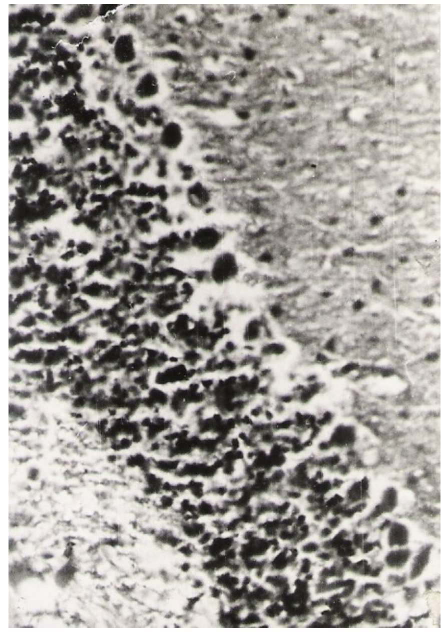

Fig.3

Fig.4

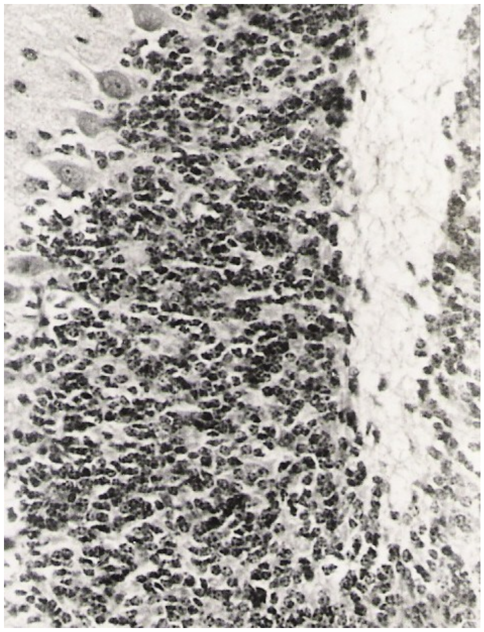

Figure 1. Photomicrograph of cerebellar folia of 2 week old mice 7 day p.i. showing degenerating Purkinje cells with Pyknotic nuclei. X20
Figure 2. Photomicrograph of cerebellar folia of 3 week old mice 7 day p.i. showing many pyknotic and disintegrating Purkinje cells. The molecular layer shows signs of degeneration. X20
Figure 3. Photomicrograph of cerebellar folia of 5 week old mice 7 day p.i. showing elongated Purkinje cells with vacuolation of the connective tissue. X20
Figure 4. Photomicrographs of cerebellar folia of 6 week old mice7 day p.i. showing dark type Purkinje cells after exposure to 111kBq/gm body weight HTO. X40